AMENDMENT TO THE SPECIFICATION

Kindly amend the title of the application to read as follows:

METHODS ~~METHOD~~ AND COMPOSITIONS UTILIZING DIFFERENTIAL SPLICING EVENTS IN BLOOD CELLS FOR ~~DETECTING~~ THE REMOTE DETECTION OF PATHOLOGICAL EVENTS

Kindly amend the specification on page 10, line 16, through page 11, line 9, as follows:

The nucleic acid populations used to obtain clones or to constitute banks are, for example, RNA (total or messenger RNA) of cells extracted from a pathological situation and RNA (total or messenger RNA) corresponding to a control situation, or nucleic acids derived from this total or messenger RNA by reverse transcription, amplification, cloning into vectors, etc. These nucleic acids can be prepared according to methods familiar to those skilled in the art. Briefly, these methods generally comprise lysis of the cells, tissue or sample, and isolation of the RNA by extraction. In particular, this can consist of a treatment with chaotropic agents such as guanidium thiocyanate (which destroys the cells and protects the RNA) followed by extraction of the RNA with solvents such as phenol or chloroform. Those skilled in the art are familiar with such methods (see Maniatis et al., Chomczynski et al., Anal. Biochem. 162 (1987) 156, which is incorporated by reference), which can be easily implemented by using commercially available kits such as the US73750 kit (Amersham) for

total RNA. The RNA used does not have to be perfectly pure, and in particular the presence in the preparation of traces of genomic DNA or other cellular components such as protein, etc. is not a problem so long as they do not significantly affect RNA stability. Furthermore, in an optional manner, it is possible to use preparations of messenger RNA in place of total RNA preparations. The messenger RNA can be isolated either directly from the biological sample or from the total RNA by means of polyT sequences according to conventional methods. In this regard, messenger RNA can be obtained through the use of commercially available kits such as the US72700 kit (Amersham). The RNA can also be obtained directly from banks or from other samples prepared in advance and/or available in collections, and stored under suitable conditions.

Kindly amend the specification on page 13, lines 1-7, as follows:

This process is based on the recognition of a signature associated with each cDNA by using restriction enzymes and oligonucleotide adaptors. This label corresponds to a part of the cDNA sequence (10 nucleotides long so as to unambiguously identify the corresponding cDNA). The labels are then assembled for sequencing and analysis (Velculescu et al., Science, 1995, 270: 484-487, which is incorporated by reference). This approach therefore represents a short-cut to systematic sequencing.

Kindly amend the specification on page 13, line 17, through page 14, line 3, as follows:

This method makes use of an oligo-dT primer and random primers to perform PCR on cDNA populations. The PCR products are then compared on very high resolution gels. Differentially expressed fragments are then isolated and their presence confirmed by northern blot analysis prior to sequencing.

Several variants of this method have been described (Prashar and Weissman, PNAS, 1996, 93: 659-663, which is incorporated by reference). These variants differ in terms of the primer and restriction enzymes and adaptor used. As with the SAGE method, they make use of the 3'-ends of cDNAs. This approach is made accessible by the existence of several commercially available kits.

Subtractive cloning

This method is based on the elimination of cDNAs that are common to the two samples under comparison. Thus, different kits in which the "tester" cDNA is hybridized with an excess of "driver" cDNA are available (Clontech). The final product consists of a pool of PCR-amplified fragments derived from differentially expressed cDNAs, which can be cloned in a suitable vector for subsequent analysis. RDA (Representational Difference Analysis) is another method based on this principle of subtraction (Lisitsyn et al., Science, 1993, 259: 946-951, which is incorporated by reference).

Kindly amend the specification on page 15, lines 9-19, as follows:

- Spinal muscular atrophy is one of the most common genetic diseases. Two genes, SMN1 and SMN2, encode identical proteins. Loss of the two SMN1 alleles and a splicing mutation in the SMN2 gene lead to disease development (Lorson et al. Proc. Natl. Acad. Sci. USA 1999, 96: 6307-6311, which is incorporated by reference).
- Specific splicing mutations in the presinillin gene, PS1, have been found in the biopsy specimens of patients with Alzheimer's disease (Isoe-Wada et al. Eur. J. Neurol., 1999 : 163-167, which is incorporated by reference)
- The glutamate transport protein is of major importance in neurodegenerative diseases such as amyotrophic lateral sclerosis or epilepsy, for example. Splicing mutations in this transporter affect its function (Meyer et al . Neurosci. Lett, 1998. 241: 68-70, which is incorporated by reference).

Kindly amend the specification on page 17, lines 1-22, as follows:

- macrophage "scavenger" receptors are membrane glycoproteins required for the physiological and pathological response of these blood cells and their functions are regulated by isoforms generated by splicing (Gough et al. J.Lipid Res ; 1998 ; 39: 531-543, which is incorporated by reference.)

-Activation of T lymphocytes requires the functional presence of several regulatory proteins and receptors. Boriello et al. (J. Immunol. 1995 ; 155: 5490-5497, which is incorporated by reference) reported the presence of isoforms of the B7 activation cofactor, generated by alternative splicing of this gene, thus underscoring the considerable plasticity that these splicing variants confer to the immune response.

To take into account these phenomena and this complexity, and to thereby isolate signatures that are specific to a pathological state and present in blood cells, the process of the invention advantageously makes use of splicing events characteristic of situations of deregulation, as genetic markers.

To do so, the present invention uses, for example, differential qualitative nucleic acid banks produced according to "DATAS" methodology described in ~~the unpublished~~ international patent application PCT/FR 99/00547, which is incorporated by reference. In particular, such banks can be prepared by hybridization between the nucleic acid population derived from cells isolated from the blood in a pathological situation, and the nucleic acid population derived from circulating cells in the control situation, and isolation, from the hybrids formed, of the nucleic acids corresponding to differential splicing.

Kindly amend the specification on page 18, lines 17-24, as follows:

According to a specific embodiment of the invention, the hybridization is carried out in a phenol emulsion, for example according to the PERT method ("Phenol Emulsion DNA

Reassociation Technique") described by Kohne D.E. et al. (Biochemistry, Vol. 16, No. 24, pp 5329-5341, 1977, which is incorporated by reference). The hybridization is advantageously ~~avantageously~~ carried out in a phenol emulsion maintained by thermocycling (temperature increase from approximately 37°C to approximately 60/65°C) and not by agitation, according to the method described by Miller and Riblet (NAR 23 (1995) 2339, which is incorporated by reference).

Kindly amend the specification on page 19, lines 17-19, as follows:

It is understood that other specific variants and conditions for the isolation of nucleic acids, hybridization and obtaining of qualitative clones, are indicated in International ~~the not-yet-published~~ application No. PCT/FR99/00547, which is incorporated by reference.

Kindly amend the specification on page 20, lines 6-14, as follows:

The banks provided for by the invention can comprise 10 to 50,000 clones, more generally 10 to 10,000 clones, and even more preferably 50 to 5,000 clones. The clones are generally deposited in a well-ordered fashion on one or more supports so as to facilitate analysis of the hybridization results. The support can be composed of glass, nylon, plastic, fiber, etc. or generally be any solid support suitable for the deposit of nucleic acids. The banks can be

deposited on the supports by conventional methods known to those skilled in the art, as described for example in the international application No. PCT/FR99/00547, which is incorporated by reference.

Kindly amend the specification on page 26, line 9, through page 27, line 6, as follows:

The murine hepatocarcinoma (HCC) model is linked to the restricted expression in liver of the SV40 virus early sequences encoding the large and small T antigens (Dubois, N., Bennoun, M., Allemand, I., Molina, T., Grimber, G., Daudet-Monsac, M., Abelanet, R., and Briand, P. (1991) Time course development of differentiated hepatocarcinoma and lung metastasis in transgenic mice. J. Hepatol., 13, 227-239, which is incorporated by reference). The transgene is under the control of the human antithrombin III promoter which drives early, continuous expression of the viral antigens. For this reason, hepatocellular proliferation undergoes a two-step perturbation. The proliferation index of the transgenic hepatocytes is proportionally higher than normal during liver development (from birth to 5 weeks), and then appreciably decreases, without however reaching the low levels characteristic of normal, quiescent liver. These transgenic mice systematically develop differentiated HCC that are fatal to all animals before 7 months. Despite an early deregulation of hepatocyte proliferation, hepatomegaly occurs only late in the course. Analysis of the preneoplastic steps preceding development of HCC has revealed the existence of an apoptotis compensating mechanism that maintains normal liver mass in this model (Allemand et al., 1995). It is noteworthy that this apoptosis stops at the very moment when the normal liver enters quiescence. Beyond this point, it appears that hepatic homeostasis is no longer controlled. A systematic study of sensitivity to apoptosis showed that the hepatocytes derived from this

transgenic model had acquired resistance to cell death which was dependent on the CD95/Fas system (Rouquet N, Allemand I, Molina T, Bennoun M, Briand P and Joulin V. (1995) Fas-dependent apoptosis is impaired by SV40 T-antigen in transgenic liver. Oncogene, 11, 1061-1067; and Rouquet N, Allemand I, Grimber G, Molina T, Briand P and Joulin V. (1996) Protection of hepatocytes from Fas-mediated apoptosis by a non-transforming SV40 T-antigen mutant. Cell Death & Diff., 3, 91-96; both of which are incorporated by reference) by a mechanism independent of alternative splicing of the CD95/Fas receptor. However, only a global analysis of splicing alterations can explain an alteration in this process for all the players involved in the CD95/Fas receptor signaling pathway.

Kindly amend the specification on page 27, lines 25-32, as follows:

These cDNAs are used to monitor tumor progression in this transgenic model and in a series of transgenic murine HCC models (Bennoun M, Grimber G, Couton D, Seye A, Molina T, Briand P and Joulin V. (1998) The amino-terminal region of SV40 large T antigen is sufficient to induce hepatic tumours in mice Oncogene, 17, 1253-1259, which is incorporated by reference ~~*in press*~~). Thus, by using specific cDNAs detected at different, very early stages in blood cells, before development of the tumor, it is possible to predict, in a mixed population of healthy and transgenic mice, which animals will develop a tumor.

Kindly amend the specification on page 28, lines 7-14, as follows:

In a strategy which uses the cDNA banks obtained according to the aforementioned processes of the invention, a total probe prepared from blood samples from cancer patients can also be used to screen for signatures common to the different banks established from murine models at different stages of tumor progression, on the one hand, and from biopsies of different human tumors on the other hand. These hybridizations are carried out according to methods familiar to those skilled in the art (in particular, consult the hybridization conditions set forth in International application No. PCT/FR99/00547, which is incorporated by reference).